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Nucleoprotein Autoantibodies in Lupus Erythematosus

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Autoantibodies against deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) proteins are commonly detected in patients with lupus erythematosus (LE). Antibodies against native DNA are frequently detected in a subset of LE patients with a high prevalence of renal disease. Single-stranded DNA antibodies are also commonly detected in patients with systemic lupus erythematosus (SLE) but recent evidence indicates that approximately 25% of patients with benign, cutaneous (discoid) lupus also possess single-stranded DNA IgM autoantibodies.

LE patients also frequently possess antibodies directed against a variety of ribonuclear proteins (RNP). These RNA protein autoantibodies are generally divided into two groups. One group is termed snRNPs (small nuclear ribonuclear protein); the other is termed scRNPs (small cytoplasmic ribonuclear protein). Anti-RNA protein autoantibodies occur as frequently in patients with SLE as do native DNA antibodies. Furthermore, in contradistinction to nDNA antibodies, lupus patients generally make large quantities (detected by gel precipitin techniques) of anti-RNP antibodies. The anti-RNP antibodies are directed against proteins that bind with specific RNA nucleotides. The best evidence at present indicates that these RNA proteins containing the specific RNA nucleotides are involved in RNA processing and post-translational activities such as protein synthesis. Furthermore, these SLE autoantibodies are now being employed, together with other autoantibody systems detected in other connective tissue diseases, to define the biological role of the respective RNA proteins.

The study of autoantibodies against nucleoproteins has received a great deal of renewed interest during the past 5 years. These autoantibodies are of great importance to the dermatologist and rheumatologist studying their association with, and possible pathologic role in, systemic lupus erythematosus (SLE) as well as other connective tissue diseases. The frequency of occurrence of these autoantibodies in lupus erythematosus (LE) is depicted in Table I ([1–3]; H Mogavero, D Tuffanelli, J Epstein, unpublished observations). These autoantibodies are also now employed as probes in attempts to decipher the biologic role of a relatively newly described set of small molecular weight ribonuclear proteins (RNP) found predominantly within the nucleus and to a lesser extent, the cytoplasm of eukaryotic cells [4,5]. Finally, some of these autoantibodies are of great interest to immunogeneticists and immunologists studying the regulation of autoantibody synthesis since there is evidence to indicate that some of these autoantibodies are under genetic control by genes present in or near the HLA-DR loci on the major histocompatibility complex located on the short arm of chromosome 6 [3,6,7].

"LE CELL"

From a historical viewpoint, investigations of nucleoprotein autoantibodies in SLE began with the work of Haserick et al and Hargraves et al [8,9]. These investigators described the LE cell. The LE cell is a leukocyte which has ingested large, amorphous nuclear material (DNA-histone complex). The LE cell is produced by the DNA-histone autoantibody (LE factor) binding the histone nucleoprotein complex followed by the activation of complement. The leukocyte then, via complement and Fc γ receptors, binds and phagocytizes the nucleoprotein antibody complement complex.

ANTINUCLEAR ANTIBODIES

Subsequent to the demonstration of the role of antinuclear antibodies in the formation of the LE cell, immunofluorescent techniques were developed which have provided a reliable technique for the determination of antibodies against nuclear constituents. This technique, the fluorescent antinuclear antibody test (FANA), has become the most commonly employed screening test for the diagnosis of SLE. Approximately 90% of SLE patients, using heterologous tissue i.e., mouse liver, rat kidney, etc., as antinuclear antibody substrates, demonstrate significant antinuclear antibody titers (i.e., $\geq$30). However, with the introduction of normal and malignant human cell lines as antinuclear antibody substrates (e.g., KB, hep-2, wil-2 cells), the sensitivity for the detection of antinuclear antibodies increased [10]. However, in our experience there are still some predominantly cutaneous lupus patients with systemic features who fail to give significant antinuclear antibody titers even with these newer cell culture lines. We have found, these patients frequently possess denatured DNA (ssDNA) and/or Ro antibodies (see below) [11].

In the past, specific antinuclear antibody patterns were judged to be significant. With the realization, however, that lupus patients make antibodies against a variety of cell constituents, the specific fluorescent antibody patterns appear now to be of limited value. One pattern, however, if detected, should provoke additional studies. The peripheral or shaggy pattern which denotes the presence of anti-nDNA antibodies should be confirmed with a specific anti-nDNA assay (see below).

Since the late 1940's and early 1950's, the study of these autoantibodies has dramatically progressed. At our present state of evolving knowledge, 3 types of autoantibodies appear to be of primary importance in SLE. These are DNA, RNA, and histone autoantibodies.

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Abbreviations:
BMZ: basement membrane zone
CIC: circulating immune complexes
LE: lupus erythematosus
MCTD: mixed connective tissue disease
nDNA: antibodies against double-stranded DNA
RNP: ribonuclear protein(s)
SCLE: subacute cutaneous lupus erythematosus
scRNPs: small cytoplasmic ribonuclear proteins
SLE: systemic lupus erythematosus
snRNPs: small nuclear ribonuclear proteins
SS: Sjögren's syndrome
ssDNA: denatured DNA
Z-DNA: antibodies directed against the left-handed helix variant of nDNA

TABLE I.[a] *Frequency of various autoantibodies*

Disease	ssDNA	nDNA	Sm	nRNP	Ro (SS-A)	La (SS-B)	Histones
Benign cutaneous "discoid"	25%[b]	0	0	10%	24%	3%	—
SLE	~90%	45%	20%	38%	25%	10%	33%
Drug induced lupus[c]	10%	0	0	0	0	0	90%[b]

[a] Table compiled from published and unpublished data from our laboratory and from *[2]*.
[b] γM isotope.
[c] Hydralazine and procainamide.

DNA ANTIBODIES

DNA antibodies are commonly found in SLE patients. Antibodies against ssDNA, as determined by a Farr assay, have been reported in as many as 90% of SLE patients [3]. nDNA antibodies (antibodies against double-stranded DNA) have been reported in 40–60% of SLE patients [1,2]. These nDNA antibodies, unlike ssDNA antibodies, are highly specific for SLE whereas, ssDNA antibodies are also found in other connective tissue diseases such as rheumatoid arthritis. Recently, antibodies against Z-DNA (antibodies directed against the left-handed helix variant of nDNA) have also been described in SLE patients [12].

There are 4 epitopes to which DNA antibodies are commonly directed although heterogeneity of DNA antigenic determinants are frequently noted. The 4 common DNA epitopes are: (1) Confirmational antigenic determinants involving the double-stranded helices of nDNA. These antibodies are uncommon but are specific for SLE. (2) Antigenic determinants involving the phosphodiester backbone of nDNA. Antibodies against these determinants cross react with ssDNA. These epitopes are composed of double-stranded DNA polynucleotides and synthetic double-stranded polynucleotides (e.g., poly [dA-dT]·poly [dA-dT] and poly dG·poly [dC]) are capable of binding to these antibodies but synthetic double-stranded RNA polynucleotides (e.g., poly [A]·poly [U] and poly [I]·poly [C]) do not. (3) Antigenic determinants involving purine and pyrimidine bases. Antibodies against these determinants are specific for ssDNA and don't cross react with nDNA. (4) Antigenic determinants involving Z-DNA. Antibodies reactive against Z-DNA are of 2 types. One type reacts with the purine and pyrimidine bases and, therefore, these antibodies cross react with ssDNA; the other antibody shows specificity for the Z-DNA phosphodiester backbone. These latter antibodies are uncommon but are specific for SLE.

nDNA ANTIBODIES

nDNA antibodies are highly specific for SLE and are commonly found in SLE patients with significant renal disease and hypocomplementemia Table II [13,14]. There is evidence that anti-nDNA antibodies in the form of DNA anti-DNA immune complexes are involved in the pathogenesis of the SLE glomerulonephritis. Immunofluorescent studies have detected the presence of DNA in the immune deposits in affected glomeruli and acid elution studies of the renal cortices of patients dying with glomerulonephritis have detected an enhanced concentration of anti-nDNA antibodies compared to serum [14].

Although circulating immune complexes (CIC), composed of DNA and anti-DNA and other circulating antigen antibody complexes, have been viewed as the major pathologic mechanism for the induction of glomerulonephritis and vasculitis in SLE, several new findings suggest caution in ascribing CIC to the pathogenesis of all the glomerulonephritic and vasculitic lesions seen in SLE. For example, our preliminary data employing solid phase anti-C3 and C1q-binding immune complex assays indicate no apparent correlation between the quantity of CIC, serologic subsets of lupus, and clinical features of immune complex mediated disease i.e., glomerulonephritis and vasculitis (R Hamilton, E Alexander, TT Provost, unpublished observations). Also, recent evidence indicates that SLE patients are capable of producing complement-fixing antibodies which bind cell surface antigens on vascular endothelium cells. Thus, it is conceivable that in at least some SLE patients, a Gell and Coombs type II reaction, may also be capable of inducing a significant vascular insult [15]. The data by Izui et al demonstrating that DNA, [both single-stranded (denatured) and double-stranded (native)], have a propensity to bind to collagen along the basement membrane zone (BMZ) of the glomeruli and skin also offers an alternative explanation to the CIC theory of pathogenesis [16]. Based upon Izui et al's data, it is conceivable that "in situ" immune complex formation composed of DNA, anti-DNA antibodies and not CIC plays the major pathologic role in the genesis of the glomerulonephritis. It is theorized that following the binding of the DNA to the BMZ, the anti-DNA antibody then binds to the DNA, forming an "in situ" immune complex. The "in situ" complex activates the complement sequence producing the inflammation that ultimately destroys the glomeruli. Indirect evidence to indicate that DNA does preferentially bind to the skin and BMZ in vivo, is found in studies evaluating the relationship of a positive lupus band test (immunoglobulin and complement deposition along the skin BMZ of normal appearing skin) and anti-nDNA antibodies [17,18]. Studies from several laboratories indicate a statistically significant increased prevalence of a +LBT and anti-DNA but not Sm, nRNP, Ro, etc., antibodies. Other studies have presented direct evidence for the presence of DNA antigens in some positive LBTs [19].

TABLE II. *Features associated with nDNA antibodies*

1. ↑ Prevalence of renal disease
2. Hypocomplementemia
3. Positvie lupus band test

Finally, several studies of vasculitic lesions in SLE have described 2 distinct types of histopathologic inflammatory infiltrates. The most common is a leukocytoclastic angiitis; the second type is characterized by a mononuclear perivascular infiltrate in which mononuclear cells invade the blood vessel walls [20]. Similar histopathologic infiltrates have been described in vasculitic lesions occurring in Sjögren's syndrome (SS) [21,22]. The leukocytoclastic vasculopathy is thought to be the result of CIC; the pathophysiologic mechanism responsible for the mononuclear vasculopathy, however, is unknown.

These data as recounted above, constitute a growing body of evidence suggesting that at least several possible pathophysiologic mechanisms may be responsible for SLE vasculitic and glomerulonephritic lesions.

ssDNA ANTIBODIES

ssDNA antibodies are not specific for SLE. However, ssDNA antibodies may be the only positive serologic finding in some SLE patients failing to demonstrate a significant ANA titer [11]. Many of these patients possess significant photosensitive cutaneous disease. Recent studies indicate that approximately 25% of discoid lupus patients possess ssDNA of the IgM isotype (Table III) [23]. These discoid lupus patients possessing ssDNA antibodies may be at risk to develop systemic features. In addition, acid elution studies of kidneys of SLE patients dying with glomerulonephritis, indicate an enhanced concentration of these ssDNA antibodies compared to the serum [24]. No

TABLE III. *Features associated with ssDNA antibodies*

1. Associated with photosensitive lupus dermatitis
2. IgM ssDNA antibodies detected in 25% of discoid lupus erythematosus
3. May be involved in renal disease in lupus

nDNA antibodies were detected. These studies have been interpreted as indicating that ssDNA antibodies, theoretically in the form of ssDNA, anti-ssDNA immune complexes, played a pathologic role in the genesis of the glomerulonephritis.

These studies indicate that ssDNA antibody determinations may be an important diagnostic and even prognostic test to be employed in the evaluation of discoid lupus patients or in patients suspected of having lupus but who fail to demonstrate a significant ANA titer. Since ssDNA antibodies are directed against purine and pyrimidine bases buried and sterically inaccessible in the double-stranded DNA helix, it is possible to have high titer ssDNA antibodies and yet fail to demonstrate a positive ANA titer [11].

TESTS FOR DNA

DNA antibodies are generally detected by radioimmunoassay, immunofluorescent techniques or ELISA assays. At the present time, the *Crithidea luciliae* assay is a very popular assay for the detection of nDNA antibodies (Fig 1). The kinetoplast of this hemoflagellate contains circular DNA and, thus, theoretically possesses the same antigenic determinates as nDNA. However, recent experience with this assay by Gilliam's and other groups indicate that the kinetoplast may contain other antigenic determinants and thus not be absolutely specific for nDNA as has been previously presumed [25,26]. The nature of these other antigenic determinants in the *Crithidea* kinetoplast is/are unknown but some preliminary data suggest that they may be histones.

It is important to note that the vast majority of DNA-positive patients make quantities of anti-DNA antibodies in the μg range. Thus, very sensitive techniques, i.e., radioimmunoassays, *Crithidea luciliae* and ELISA technologies have been developed [27]. Only occasional SLE patients possess significant quantities of anti-nDNA antibodies to be detected in the less sensitive gel double-diffusion assay.

RNA ANTIBODIES

The second major class of autoantibodies in SLE are directed against RNA macromolecules. Lupus patients make autoantibodies against a variety of RNA proteins. Recently, it has been determined that systemic lupus patients commonly make autoantibodies reactive against a newly described class of small molecular weight RNA proteins residing predominantly in the nucleus and to a lesser extent in the cytoplasm of the cell [4,5]. These RNA proteins contain specific RNA nucleotides (Fig 2, Table IV). These molecules are termed small nuclear ribonuclear proteins (snRNPs) and small cytoplasmic ribonuclear proteins (scRNPs), respectively. It is estimated that these ribonuclear proteins compose 0.1%–1% of the total RNA content of cells. These small ribonuclear proteins are phylogenetically highly conserved. They are capped (3-methyl guanosine at the 3′ position) or uncapped and are synthesized by either RNA polymerase II or III (Table IV). The exact function of these small ribonuclear proteins is/are unknown but the evidence at the present time indicates that these ribonuclear proteins are probably involved in RNA processing such as splicing and excision and posttranslational activities.

SM ANTIBODIES

The first autoantibody directed against these snRNPs to be described was the Sm antibody (named after an abbreviation of the surname of the first patient in whom it was initially detected) [29]. This is an autoantibody directed against a

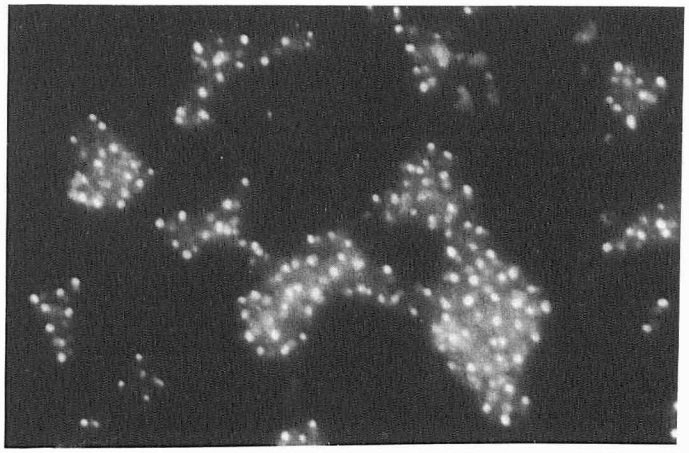

FIG 1. Positive *Crithidea luciliae* assay. *Crithidea* organisms overlayed with anti-nDNA containing lupus serum and developed with a fluorescein labelled antihuman globulin preparation. Kinetoplast containing circular DNA stains apple green.

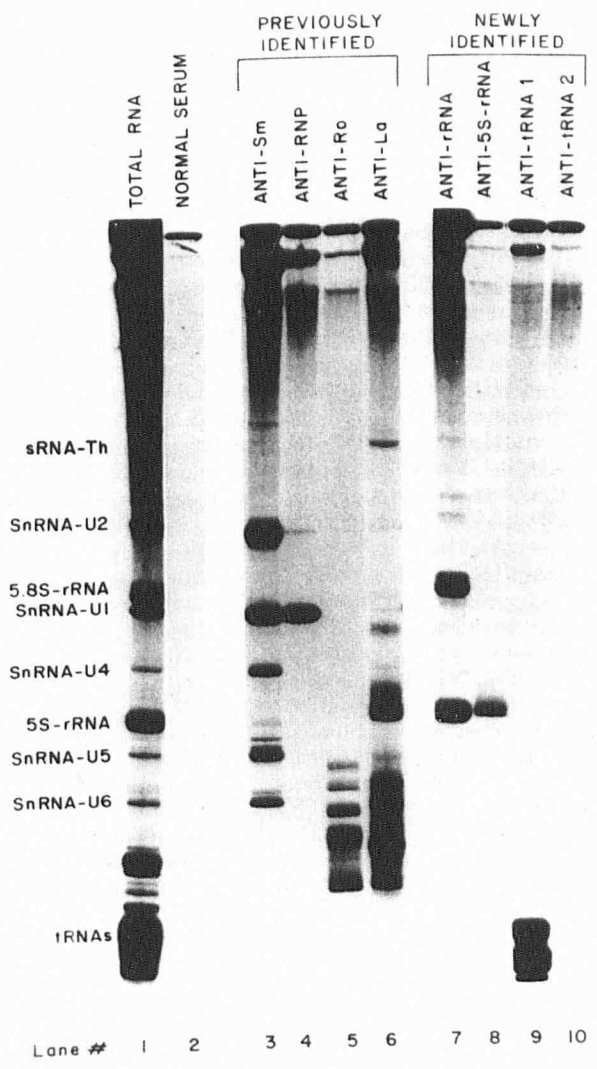

FIG 2. Polyacrylamide gel electrophoresis of small RNAs from Hela cells precipitated by autoantibodies from systemic lupus erythematosus and other patients. Hela cells were labeled with $^{32}PO_4$ and total cell extracts prepared. (*Reprinted by permission of the authors and the editor of the Journal of Clinical Investigation* [28].)

TABLE IV. *Antibodies against small ribonucleoproteins*

Antibody	Class of RNA nucleotides	Other features
Anti-nRNP	U_1	Capped with trimethylguanosine; synthesized by RNA polymerase II
Anti-Sm	U_1, U_2, U_4, U_5, U_6	All but U_6 capped with trimethylguanosine; U_6 capped by unknown nucleotide; synthesized by RNA polymerase II
Anti-Ro (SS-A)	Y_1-Y_5[a]	Noncapped; synthesized by RNA polymerase III
Anti-La (SS-B)	Many RNAs including Y_1-Y_5, VA RNAs[b] and EBER RNA[c]	Noncapped; synthesized by RNA polymerase III

[a] Cytoplasmic RNA nucleotide.
[b] VA RNAs = Viral-associated RNAs of adenovirus.
[c] EBER RNA = Epstein-Barr virus-associated RNAs.

TABLE V. *Clinical diagnoses in 43 nRNP-positive patients*

Diagnosis	Number of patients	Percentage
Mixed connective tissue disease	5	12
Progressive systemic sclerosis	1	2
Systemic lupus erythematosus	34	80
Rheumatoid arthritis	1	2
Drug-induced systemic lupus erythematosus	1	2
Nonrheumatic disease	1	2

TABLE VI. *Clinical features associated with nRNP autoantibodies*

1. Sclerodactyly
2. Sclerodermatous features
3. Raynaud's phenomenon
4. Esophageal dysmotility
5. Decreased prevalence of renal disease

snRNP containing 2 polypeptides of approximately 15,000 and 17,000 M_r [30]. The U1, U2, U4, U5, and U6 series of RNA nucleotides (U connoting uridine-rich RNA) bind to this protein. (Fig 2, Table IV). These anti-Sm antibodies are highly specific for SLE. These antibodies almost never occur alone and are almost always found in the presence of anti-nRNP antibodies. There is some evidence to suggest that these antibodies are found in a lupus patient population possessing an increased incidence of central nervous system and renal disease [31].

nRNP ANTIBODIES

Antibodies directed against nRNP are directed against a snRNP (approximately 85,000 daltons) containing the U1RNA nucleotide (Fig 2) [30]. The U1RNA protein complex is the most plentiful of the snRNPs (~10^6 copies/cell). Recent evidence employing anti-nRNP and anti-Sm antibodies as probes indicates that the U1RNA may play a significant role in the excision and splicing process in the formation of messanger RNA. These autoantibodies, both of which are directed against proteins containing the U1RNA, block this process [32].

The nRNP(U1 snRNP) antibodies are found predominantly in a patient population satisfying the American Rheumatism Association (ARA) criteria for the diagnosis of SLE (Table V) [33]. However, there is a definite small group of patients possessing nRNP antibodies who have clinical features of scleroderma, lupus, and dermatomyositis. These patients have been termed the mixed connective tissue disease (MCTD) syndrome [36]. The common clinical features of the MCTD syndrome are listed in Table VI. Some investigators ascribe to all nRNP positive patients, the designation of the MCTD. These nRNP patients, however, are not homogeneous in clinical presentation and clinically may change with the progression of time. For example, a long-term follow up of the original nRNP-positive MCTD patients has indicated that a majority of these patients have evolved into a clinical picture of scleroderma or SLE. I believe all would agree, however, that this group of nRNP-positive patients have a significantly lower prevalence of clinical renal disease than the nDNA-positive lupus patients and, thus, a better prognosis.

LA ANTIBODIES

Antibodies against the La antigen are directed against a snRNP containing a heterogeneous group of RNA nucleotides including RNA nucleotides of the y1–y5 (cytoplasmic) series as well as RNA nucleotides of adenovirus (VA-RNA) and the Epstein-Barr virus [5,36] (Table IV). Molecular weight studies of the La protein employing polyacrylamide gel electrophoresis (PGE) by several laboratories indicate an approximate 41,000

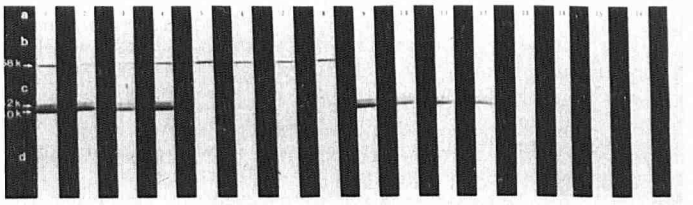

FIG 3. Immunoblotting analysis of partially purified human spleen Ro/La antigens and a representative panel of SSA/Ro and SSB/La sera (*lanes 1–4*), SSA/Ro sera (*lanes 5–8*), SSB/La sera (*lanes 9–12*), and normal human sera (*lanes 13–16*). The SSA/Ro antisera reacted with a protein band of 58k M_r (*shown by arrow*), the SSB/La antisera reacted with 2 close bands of 40k and 42k M_r (*shown by arrow*), respectively. Antisera with SSA/Ro and SSB/La specificities reacted with both groups of proteins. Normal human sera did not show reactivity. Molecular weight standards are listed on the *left of lane 1* as follows: (*a*) phosphorilase (94k, (b) albumin (68k), (c) ovalbumin (45k), and (d) soybean trypsin inhibitor (20k).

dalton molecular weight (Fig 3) [37,*]. The La antibody (named after an abbreviation of the surname in whom this antibody was first detected), is immunologically identical to the SS-B and SjT antibody systems described in SS [38,39]. This antibody system is almost always found together with the Ro(SS-A) antibody system (see below). Anti-La antibodies are seen in approximately 10% of SLE patients. This antibody system is almost never seen in the normal patient population.

RO ANTIBODIES

The Ro antibody is directed against a 58–60 kD protein present in greatest concentration in the brain and heart and is identical to the SS-A antibody systems described in SS (Fig 3) [36,40,41]. This antibody system is also probably identical to the SjD and Ha antibody systems [39]. The Ro antibody system has proved most important in the evaluation of lupus patients possessing prominent photosensitive cutaneous lupus lesions. In addition to prominent photosensitive lupus lesions, these Ro-positive lupus patients frequently are rheumatoid-factor positive [11,13] and may have SS. Most important, these Ro-positive patients have a low prevalence compared to nDNA positive lupus of renal disease (Table VII). Ro-positive lupus patients have been described under the heading of ANA-negative SLE [11], subacute cutaneous lupus erythematosus (SCLE) [7], homozygous C2 and C4 deficient associated with a lupus-

* Herrera-Esparza R, Halim HY, Provost TT, Diaz LA: A sensitive and specific assay to detect SSA/Ro and SSB/La antibodies. In press.

TABLE VII. *Clinical and laboratory features of Ro-positive SLE patients*

1. Photosensitive lupus dermatitis
2. Sjögren's syndrome
3. Rheumatoid factor
4. Low prevalence of renal disease

TABLE VIII. *Ro(SS-A) antibody clinical associations*

1. ANA negative SLE approximately (70%)
2. Subacute cutaneous lupus erythematosus (SCLE) (approximately 70%)
3. C_2 deficient lupus-like disease (75%)
4. C_4 deficient lupus-like disease (50%)
5. Neonatal lupus erythematosus (approximately 100%)
6. Aged lupus patients (approximately 90%)
7. Japanese (? all Oriental) lupus patients (50%)
8. Sjögren's syndrome patients (40%)

(%) Percent of patients with particular clinical designation who are Ro(SS-A)-positive.

like syndrome [42], and neonatal lupus [43,44] (Table VIII). These patients all have in common the presence of an intense photosensitive (many of these patients burn through window glass) cutaneous lupus dermatitis. In our experience with approximately 150 Ro-positive lupus patients, 40% of these patients have a distinctive, annular, polycyclic lupus dermatitis demonstrating sparse scaling and little, if any, scar formation. Because these lesions occur in a predominantly cutaneous lupus patient population, who at least 50% of the time satisfy predominantly the minor criteria for the diagnosis of SLE, these patients have been aptly termed by Sontheimer and Gilliam as having SCLE [7].

Most recent data indicate there is a striking increased prevalence of the Ro antibody in lupus patients with the onset of their disease process after the age of 55. Other recent studies indicate that the Ro antibody occurs much more frequently in Japanese (50%) than in Occidental (25%) lupus patients. The reason(s) for these statistically significant associations of the Ro antibody with elderly and Japanese (and perhaps all Oriental lupus patients) is/are unknown.

Perhaps most importantly, the Ro and La antibodies have been described to occur in almost all mothers and infants with the neonatal lupus syndrome [45,46]. These studies have demonstrated that the mother produces these antibodies and these antibodies pass across the placenta to the affected infant who may die in utero, be born with isolated congenital heart block [47], or in its most benign form, demonstrate a lupus dermatitis (most commonly, SCLE). The Ro antibody and lupus dermatitis generally disappear by 6 months of age. The congenital heart block, however, is persistent. At present, the pathophysiologic mechanism(s) operative in the genesis of the skin and heart disease in the infant is/are unknown. These data, however, constitute the best evidence at the present time that the Ro and La antibodies are capable of inducing clinical features of lupus erythematosus.

In addition to a prominent association with cutaneous lupus patients, the Ro antibody as detected by a very sensitive ELISA assay, has been found to occur in almost all SS patients. Approximately 40–45% of SS patients demonstrate the Ro antibody by gel double-diffusion (large quantities of Ro antibody). SS patients frequently demonstrate evidence of cutaneous vasculitis [22]. The 2 most common presentations of cutaneous vasculitis are palpable purpura of the lower extremities (this dermatitis is indistinguishable from Waldenstrom's hyperglobulinemia) and urticaria-like vasculitis. The vasculitis, associated with high titers of the Ro antibody (ELISA test positive; gel double-diffusion positive), is generally a leukocytoclastic angiitis and is frequently accompanied by rheumatoid factor activity. The vasculopathy occurring in low-titered Ro-positive SS patients (ELISA test positive, gel double-diffusion negative) is generally characterized as a mononuclear vasculopathy. The most important clinical observation associated with the cutaneous vasculitis in SS is that approximately 60% of these vasculitic SS patients also have evidence of central and peripheral nervous system disease. Evidence suggests that the central and peripheral nervous disease is also vasculitic in origin.

RNA ANTIBODY TESTING

Tests for antibodies against the RNA macromolecules are most commonly performed employing gel double-diffusion. In the past, hemagglutination techniques using RNAase had been employed to detect RNA-sensitive extractable nuclear antigen (ENA) antibodies. ELISA assays for all these RNA antibodies will probably be soon available.

From a conceptual point of view, it is intriguing to observe that lupus patients make relatively large quantities of anti-RNA antibodies (mgm) which can readily be detected in gel double-diffusion. Considering the previous bias on the part of the immunologic community regarding the importance of the anti-DNA antibodies in lupus, it is interesting to note that lupus patients make anti-RNA antibodies as frequently as they do anti-DNA antibodies and generally make much larger quantities (Table I).

HISTONE ANTIBODIES

The last antibody system of importance in SLE is antihistone antibodies. Histones represent a phylogenetically, highly conserved class of basic proteins which readily bind to DNA and are involved in packing the DNA helix. There are 5 classes of histones (H1, H2A/H2B, H3, and H4). The H2A/H2B, H3, and H4 histones are wrapped by segments of approximately 146 base pairs of DNA (nucleosomes). The H1 histone binds to DNA segments linking the individual nucleosomes.

Approximately 30% of SLE patients possess antihistone antibodies [2]. However, greater than 90% of hydralazine and procainamide drug-induced lupus patients possess antihistone antibodies. Interestingly, procainamide-induced lupus patients preferentially make antibodies against the H2A/H2B histones whereas hydralazine-induced lupus-like patients demonstrate a preference for the production of H3, H4 histone antibodies. The mechanism whereby these drugs induce this lupus-like state is unknown although there is some evidence to indicate these drugs may bind to DNA. In addition, hydralazine-induced lupus patients appear to have a number of characteristics [47]. These are: (1) hydralazine dosage of greater than 300 mgm/day; (2) slow acetylators (hepatic acetyltransferase); (3) predominance of females versus males (4:1); and, (4) a statistically significant increased prevalence of the HLA-DR4 phenotype.

HISTONE ANTIBODY TESTING

Histone antibodies can be detected employing a multistep immunofluorescent procedure in which the test serum is first titered [2]. The substrate is then washed with 0.1 HCl buffer which removes the histones; the test serum is then titered on this histone-free substrate; the histone-free substrate is then reconstituted with histones and the test serum titered again.

In addition to the lupus-like syndromes induced by hydralazine and procainamide, D-penicillamine has also been shown to induce a lupus-like disease process [48]. In marked contrast to the lupus-like disease induced by hydralazine and procainamide, the penicillamine-induced lupus patients develop lupus dermatitis nDNA antibodies and glomerulonephritis.

HLA STUDIES

HLA studies indicate that high binding titers of ssDNA and nDNA may be associated with a statistically significant in-

TABLE IX. *Relationship of Ro and La antibody responses to HLA-DR phenotypes*

HLA-DR phenotype	Ro (+)	Ro (−)	*p* Value
2	60%	37.8%	.04
3	52.5%	2.6%	.0018
	La (+)	La (−)	*p* Value
2	35.7%	47%	NS
3	71.4%	27%	.0034

creased prevalence of the HLA-DR2 phenotype. No HLA association, however, was detected with either the presence of Sm or nRNP antibodies.

The Ro antibody occurs in a statistically significant association with the HLA-DR2 and DR3 phenotypes [3,6,7]. In fact, one study indicates that the Ro antibody occurred 95% of the time with either the HLA DR2 or DR3 phenotypes. The La antibody, however, was found to occur in a statistically significant association with the HLA-DR3 and not the HLA-DR2 phenotype [49, 50] (Table IX).

HLA studies of neonatal lupus infants and their mothers and normal babies of Ro-positive mothers have demonstrated that the neonatal lupus syndrome occurs on a maternal, genetic background of HLA-DR3, MB2, whereas, the maternal, genetic background of the Ro-positive mothers of normal children is HLA-DR2; MB1 [47,48]. The infants don't demonstrate an increased expression of any one phenotype. These studies strongly suggest a factor produced by the HLA-DR3 but not DR2 mothers is involved in the pathogenesis of the neonatal lupus syndrome. These studies are also important because they demonstrate a striking association of the expression of Ro and to a lesser extent, the La antibodies with the maternal HLA-DR3 phenotype but the clinical manifestations of the lupus syndrome i.e., cutaneous lupus lesions and cardiac conduction in the infant are not HLA-linked.

This brief account of the current research occurring in autoantibodies in lupus indicates a broad degree of activity and the promise of new and exciting findings.

REFERENCES

1. Synkowski DR, Reichlin M, Provost TT: Serum autoantibodies in systemic lupus erythematosus and correlation with cutaneous features. J Rheumatol 9:380–385, 1982
2. Tan EM: Autoantibodies to nuclear antigens: their immunobiology in medicine. Adv Immunol 33:167–217, 1982
3. Ahearn JM, Provost TT, Dorsch CA, Stevens MB, Bias WB, Arnett FC: Interrelationships of HLA-DR, MB, and MT phenotypes, autoantibody expression, and clinical features in systemic lupus erythematosus (SLE). Arthritis Rheum 25:1031–1040, 1982
4. Lerner MR, Steitz JA: Antibodies to small nuclear RNAs complexed with proteins are produced by patients with systemic lupus erythematosus. Proc Natl Acad Sci USA 76:5495–5499, 1979
5. Lerner MR, Boyle JA, Hardin JA, Steitz JA: Two novel classes of small ribonuclear proteins detected by antibodies associated with lupus erythematosus. Science 211:400–402, 1981
6. Bell DA, Maddison RJ: Serologic subsets and systemic lupus erythematosus: an examination of autoantibodies in relationship to clinical features of disease and HLA antigens. Arthritis Rheum 23:1268–1273, 1980
7. Sontheimer RD, Thomas JR, Gilliam JN: Subacute cutaneous lupus erythematosus a cutaneous marker for a distinct lupus erythematosus subset. Arch Dermatol 115:1409–1415, 1979
8. Haserick JR, Bortz DW: A new diagnostic test for acute, disseminated lupus erythematosus. Cleve Clin Q 16:158–161, 1949
9. Hargraves MM, Richmond H, Morton R: Presentation of two bone marrow elements: the "tart" cell and "LE" cell. Proc Staff Meet Mayo Clinic 23:25–28, 1948.
10. Harmon CE, Deng JS, Peebles CL, Tan EM: The importance of tissue substrate in SSA/Ro antigen antibody system. Arthritis Rheum 27:166–173, 1984
11. Maddison PJ, Provost TT, Reichlin M: Serological findings in patients with "ANA" negative systemic lupus erythematosus. Medicine (Baltimore) 60:87–94, 1981
12. Lafer EM, Valle RPC, Moller A, Nordheim A, Schur PH, Rich A, Stollar BD: Z-DNA specific antibodies in human systemic lupus erythematosus. J Clin Invest 71:314–321, 1983
13. Schur PH, Sandson J: Immunologic factors and clinical activity in systemic lupus erythematosus. N Engl J Med 278:533–538, 1968
14. Koeffler D, Agnello V, Carr RI, Kunkel HG: Anti-DNA antibodies and renal lesions of patients with systemic lupus erythematosus. Transplant Proc 1:933–938, 1969
15. Clines DB, Lyss AP, Reeber M, Bina M, DeHoratius RJ: Presence of complement fixing anti endothelial cell antibodies in systemic lupus erythematosus. J Clin Invest 73:611–625, 1984
16. Izui S, Lambert P, Meischer PA: In vitro demonstration of a particular affinity of glomerular basement membrane and collagen for DNA. A possible basis for a local formation of DNA—anti DNA complexes in systemic lupus erythematosus. J Exp Med 144:428–444, 1976
17. Gilliam JN, Cheatum DE, Hurd E, Stastney P, Ziff M: Immunoglobulin in clinically uninvolved skin in stystemic lupus erythematosus. J Clin Invest 53:1434–1439, 1974
18. Provost TT, Andres GA, Maddison P, Reichlin M: Lupus band test in untreated SLE patients: correlation of immunoglobulin deposition in the skin of extensor forearm with clinical renal disease and serological abnormalities. J Invest Dermatol 74:407–412, 1980
19. Tan EM, Kunkel HG: An immunofluorescent study of the skin lesions in systemic lupus erythematosus. Arthritis Rheum 9:37–46, 1966
20. O'Loughlin S, Schroeter AL, Jordon RE: Chronic urticaria-like lesions in systemic lupus erythematosus. Arch Dermatol 114:879–883, 1978
21. Soter NA, Mihm MC Jr, Gigli I, Dvorak HF, Austen FK: Two distinct cellular patterns in cutaneous necrotizing angiitis. J Invest Dermatol 66:344–350, 1976
22. Alexander EL, Provost TT: Cutaneous manifestations of primary Sjögren's syndrome: a reflection of vasculitis and association with anti Ro(SS-A) antibodies. J Invest Dermatol 80:386–391, 1983
23. Kulick K, Provost TT, Reichlin M: Antibodies to ssDNA in discoid lupus patients. Arthritis Rheum 25:639–646, 1982
24. Koffler D, Agnello V, Kunkel HG: Polynucleotide immune complexes in serum and glomeruli of patients with lupus erythematosus. Am J Pathol 74:109–114, 1974
25. Steinmetz SE, Deng JS, Rubin RL, Sontheimer RD, Gilliam JN: Reevaluation of specificity of *Crithidea luciliae* kinetoplast as a substrate for detecting antibodies to double stranded deoxyribonucleic acid. J Am Acad Dermatol 11:490–493, 1984
26. McGuigan L, Edmonds J, Wellings G, Jones M: The significance of discrepant Farr and *Critidea luciliae* tests. J Rheumatol 11:172–174, 1984
27. Harmon CE: The specificity of kinetoplast immunofluorescence. Arthritis Rheum 27:S40, 1984
28. Hardin JA, Rahn DR, Shen C, Lerner MR, Wolin S, Rosa M, Steitz JA: Antibodies from patients with connective tissue disease bind specific subsets of cellular RNA protein particles. J Clin Invest 70:141–147, 1982
29. Tan EM, Kunkel HG: Characteristics of soluble nuclear antigen precipitating with sera of patients with systemic lupus erythematosus. J Immunol 96:446–451, 1966
30. Eisenberg RA, Klapper DG, Cohen PL: The polypeptide structure of the Sm and RNP nuclear antigens. Mol Immunol 20:187–195, 1983
31. Munves EF, Schur PH: Antibodies to Sm and RNP. Prognosticators of the disease involvement. Arthritis Rheum 26:848–853, 1983
32. Yang VW, Lerner MR, Steitz JA, Flint SJ: A small nuclear ribonuclearprotein is required for splicing of adenoviral early RNA sequences. Proc Natl Acad Sci USA 78:1371–1375, 1982
33. Maddison PJ, Mogavero H, Reichlin M: Patterns of clinical disease associated with antibodies to nuclear ribonucleoprotein. J Rheumatol 5:401–411, 1978
34. Sharp GC, Irvin WS, Tan EM, Gould RG, Holman HR: Connective tissue disease: an apparently distinct rheumatic disease syndrome associated with a specific antibody to an extractable nuclear antigen (ENA). Am J Med 52:148–158, 1972
35. Nimelstein S, Brody S, McShane D, Holman HR: Mixed connective tissue disease: a subsequent evaluation of the original 25 patients. Medicine (Baltimore) 59:239–248, 1980
36. Mattioli N, Reichlin M: Heterogeneity of RNA protein antigens reactive with sera of patients with systemic lupus erythematosus. Arthritis Rheum 17:421–426, 1974
37. Venebles PJW, Smith PR, Maini RN: Purification and characterization of Sjögren's syndrome A & B antigens. Clin Exp Immunol 54:731–738, 1983
38. Alspaugh M, Maddison P: Resolution of the identity of certain antigen antibody systems in systemic lupus erythematosus and Sjögren's syndrome: an interlaboratory collaboration. Arthritis Rheum 22:796–798, 1979

39. Anderson JR, Gray KG, Beck JS, Kinnear WF: Precipitating autoantibodies in Sjögren's syndrome. Lancet 2:456–460, 1961
40. Yamagata H, Harley JB, Reichlin M: Molecular properties of the Ro/SSA antigen and enzyme-linked immunosorbent assay for quantitation of antibody. J Clin Invest 74:625–633, 1984
41. Wolin SL, Steitz JA: The Ro small cytoplasmic protein and its binding site on the Ro RNAs. Proc Natl Acad Sci 81:1996–2000, 1984
42. Provost TT, Arnett FC, Reichlin M: C2 deficiency, lupus erythematosus and anticytoplasmic Ro(SSA) antibodies. Arthritis Rheum 26:1279–1282, 1981
43. Franco HL, Weston WL, Peebles C, Forstot SL, Phanuphals P: Autoantibodies directed against sicca syndrome antigens in the neonatal lupus syndrome. J Am Acad Dermatol 4:67–72, 1981
44. Kephart DC, Hood AF, Provost TT: Neonatal lupus erythematosus: new serologic findings. J Invest Dermatol 77:331–333, 1981
45. Watson RM, Lane AT, Bias WB, Barnett NK, Arnett FC, Provost TT: Neonatal lupus erythematosus: clinical, serologic and HLA studies and a review of the literature. Medicine (Baltimore) 63:362–378, 1984
46. Lee LA, Bias WB, Arnett FC, Huff JC, Norris DA, Harmon C, Provost TT, Weston WL: Immunogenetics of the neonatal lupus syndrome. Ann Intern Med 99:592–596, 1983
47. Scott JS, Maddison PJ, Taylor PV, Esscher E, Scott O, Skinner RP: Connective tissue disease antibodies to ribonucleoprotein and congenital heart block. N Engl J Med 309:209–212, 1983
48. Batchelor JR, Welsh KI, Tinoco RM, Dollery CT, Hughes GRV, Bernstein R, Ryan P, Naish PF, Aber GM, Bing RF, Russell GI: Hydralazine-induced systemic lupus erythematosus: influence and HLA-DR on sex susceptibility. Lancet 1:1107–1109, 1980
49. Chalmers A, Thompson D, Stein HE, Graham Reid MB, Patterson AC: Systemic lupus erythematosus during penicillamine therapy for rheumatoid arthritis. Ann Intern Med 97:659–663, 1982
50. Wilson RW, Provost TT, Bias WB, Alexander EL, Edlow DW, Hochberg MC, Stevens MB, Arnett FC: Sjögren's syndrome: influence of multiple HLA-D region alloantigens on clinical and serologic expression. Arthritis Rheum 27:1245–1253, 1984